SCIENTIFIC NOTE

Color Polymorphism and Allele Frequency in a Brazilian Population of the Sunflower Caterpillar *Chlosyne lacinia saundersi* (Doubleday) (Lepidoptera: Nymphalidae)

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Polimorfismo de Coloração e Freqüência de Alelos em uma População Brasileira de Lagarta-do-Girassol *Chlosyne lacinia saundersi* (Doubleday) (Lepidoptera: Nymphalidae)

RESUMO - A freqüência de alelos de polimorfismo de coloração da lagarta-do-girassol, *Chlosyne lacinia saundersi* (Doubleday) foi calculada em uma população, com o objetivo de usar o polimorfismo como marcador genético para comparar populações de diferentes regiões ou oriundas de diferentes hospedeiros. Existem três fenótipos condicionados por dois locos interagindo epistaticamente: *rufa*, lagartas alaranjadas; *bicolor*, lagartas pretas com listras dorsais alaranjadas e *nigra*, lagartas pretas, às vezes com pontuações amareladas, sendo que esse polimorfismo torna-se bem visível a partir do quarto estádio.O mecanismo genético desse polimorfismo é bem conhecido. As amostras foram obtidas de modo independente na tentativa de se incluirem todos os cruzamentos entre os genótipos. Utilizou-se o teste de Qui-Quadrado (χ^2) para estimar com precisão o tamanho ideal da amostra. A partir de 800 espécimes de quarto e quinto ínstares, as freqüências estabilizaram. As freqüências gênicas foram calculadas com base na freqüência de cada fenótipo. O alelo R do locus *rufa* ocorreu na freqüência de 7% e o alelo r teve freqüência de 93 %. O alelo B do locus *bicolor* teve freqüência de 31,8% e o alelo b teve freqüência em torno de 62,2%.

PALAVRAS-CHAVE: Epistasia, genética de populações, genética ecológica, fenótipo

ABSTRACT - The aim of this work was at calculating allele frequency color polymorphism in a population of sunflower caterpillar, *Chlosyne lacinia saundersi* (Doubleday) from Londrina, Paraná State, Brazil. Allele frequency in insect populations can be used as genetic marker to compare populations from different geographical and host origins. There are three phenotypes conditioned by two loci interacting epistatically. The phenotypes are: *rufa* (oranged colored larvae), *bicolor* (black larvae with dorsal orange stripes) and *nigra*, larvae with the body entirely black, sometimes with dorsal yellow dots, best seen in the fourth and fifth instars. Samples were taken independently in an attempt to obtain all combinations of crossing among genotypes. The genetic mechanism of this polymorphism is well known. A Chi-Square test (χ^2) was used to estimate the ideal sample size. The frequencies stabilized, with over 800 fourth and fifth instars larvae even with increased sample size. The allele frequencies were calculated based on the frequency of each phenotype. The allele R of *rufa* locus had a frequency near 7.0% and the allele r near 93.0%, the allele B (*bicolor*) had a frequency around 31.8% and allele b frequency near 68.2%.

KEY WORDS: Epistasis, population genetics, ecological genetics, phenotype

Polymorphism is defined as the existence of several phenotypes within populations, where the most rare reaches a frequency greater than 1%. Phenotypes with different coloration patterns from different genotypes are classical examples of morphological polymorphism (Ford 1970).

The importance of coloration in ecological relationships of living beings with their environment is well known. Coloration is important in aspects, like body temperature regulation, intraspecific and interspecific communication and selection by predators (Endler 1978, Brakefield 1985). Price (1975) pointed out that phenotypic diversity aims at reducing intraspecific competition by broadening the exploitation pattern with different niches occupied by different forms.

Larvae of the last three stages of *Chlosyne lacinia*

saundersi (Doubleday) have three-color forms (Gorodenski 1969, as *C. lacinia*). The forms are *rufa* (orange), *bicolor* (black with dorsal orange stripes) and *nigra* (entirely black). According to Neck (1976), the forms are not uniform in coloration. The form *nigra*, can display yellow dots or yellow dots surrounded by white dots on the black background. These variants named "dot" and "super dot", respectively, could be observed in Brazilian populations, specially in northern of Paraná State (Lopes-da-Silva, pers. inf.) although very rarely. In *C. lacinia*, the inheritance of larvae coloration is conditioned by two loci (Gorodenski 1969). Neck (1973) found the same system in *C. gorgone* and proposed that the mechanism is the same as that of *C. lacinia*. The coloration of the fifth stage larvae head is variable, with two forms, orange with black, and entirely black. It is possible that the gene or genes responsible for this feature are not linked to loci that define body coloration, because all patterns showed this variation.

The color polymorphism frequency of *C. lacinia saundersi* larvae was evaluated in a sunflower field inside the EMBRAPA-CNPSo experimental area, in Londrina, Paraná, Brazil. This study was carried out in order to verify the sample size necessary to estimate polymorphism frequencies. The method used was subsequent samplings taken independently (values were not summed to the next sample), beginning from n = 100 larvae. Only larvae showing the visible polymorphism were sampled and a Chi-Square test was performed. The strategy was to determine the size sample in which the observed frequency was similar to the expected frequency in two subsequent samplings. A random event was considered when χ^2 values in two consecutives chi-square tests in three samples in sequence became not significant. This indicated what sample was necessary to give precision in estimating the frequency of phenotypes.

As the genetic mechanism was not yet studied in a Southern American population of *C. lacinia*, we assumed the same genetic mechanism. Successive samplings in the field showed that the most abundant pattern was *nigra* with a frequency near 52.9%. The most rare was *rufa* with a frequency around 14.4%; the bicolor form, showed approximately 32.7%. A sample of 800 specimens was found as a starting point for stabilization of phenotype frequencies (Table 1). In this sample size, the χ^2 became insignificant, showing that frequencies stabilized and that further increase of sample size was not necessary. With these data the allele frequencies were calculate.

The phenotypes frequencies found, in average for *rufa* 0.14, *bicolor* 0.33 and *nigra* 0.53. The gene frequency was calculated based on phenotypes frequency, according to the method presented by Mettler & Gregg (1969). For instance, *rufa*, with phenotype frequency = 0.14 has three possible genotypes (RrBB, RrBb and Rrbb) and f(R) (frequency of gene R) is 7%. The formula was: f(R) = f(p)/nA x n(R), where f(R) is the allele frequency of R within phenotype *rufa*, F(p) is the frequency of phenotype *rufa*, nA is the number of possible combination of alleles of locus *rufa* that define the phenotype (in this case = 1, only Rr) and n(R) is the number of alleles R present at locus *rufa* of this phenotype divided by number de alleles in this locus, in this case = 1/2. Therefore, f(R) = 0.14/1 x 1/2 = 7%. It means that 7% of alleles at the locus *rufa* are R and 93% of alleles of the whole population at this locus are r. All other frequencies were calculated using the same formula and frequencies of alleles in the one phenotype were summed with frequencies of another phenotype. The results are summarized in Table 2. We assumed that all populations of this species are not in Hardy-Weinberg equilibrium, perhaps the outcome of the existence of lethal genes in the dominant homozygote condition.

We concluded that it was possible to know a stable frequency of color phenotypes of *C. lacinia saundersi* larvae. Based on that, it is also possible to affirm that a special selection pressure over any phenotype is not occurring. This suggests the presence of a balanced polymorphism. It would be interesting to investigate the occurrence of balanced polymorphism of *rufa* associated with the fitness value and occupation of different niches.

Table 1. Frequencies in successive samplings of *C. lacinia saundersi* larval coloration pattern. Londrina, Paraná (June, 1997) with two degrees of freedom. S = Ordinal number of the sampling, N = sample size, R_{ef} = expected frequency of *rufa* pattern, R_{of} = observed frequency of *rufa* pattern, B_{ef} = expected frequency of *bicolor* pattern, B_{of} = observed frequency of *bicolor* pattern. N_{ef} = expected frequency of *nigra* pattern. N_{of} = observed frequency of *nigra* pattern. χ^2 = calculated Chi-Square. P = Probability.

S	N	Ref	Rof	Bef	Bof	Nef	Nof	χ^2	P
1	100	-	4	-	41	-	55	-	-
2	200	8.0	4	82.0	48	110.0	148	29.2	<0.0001
3	300	6.0	11	72.0	101	222.0	188	21.2	<0.0001
4	400	14.7	26	134.7	138	250.6	236	9.6	0.0082
5	500	32.5	22	172.5	200	295.0	278	8.8	0.0123
6	600	26.4	40	240.0	226	333.6	334	7.8	0.0202
7	700	46.7	68	263.7	252	389.6	380	10.5	0.0052
8	800[1]	77.7	101	288.0	260	434.3	439	9.8	0.0074
9	1000[1]	126.3	145	325.0	328	548.7	527	3.7	0.1572
10	1200[1]	174.0	187	394.0	392	632.0	621	1.2	0.5563

[1]χ^2 became not significant starting from the eighth sampling (n = 800 specimens), when the frequencies were repeated although the sample size was increased.

Table 2. Alleles frequencies of polymorphism in coloration of *C. lacinia saundersi* larvae. f(B) = frequency of allele B, f(b) = frequency of allele b, f(R) = frequency of allele R, f(r) = frequency of allele r, f(P) = frequency of phenotypes.

Phenotype	Genotype (s)	f(B)	f(b)	f(R)	f(r)	F(P)
Rufa	BBRr BbRr bbRr	0.0700	0.0700	0.0700	0.0700	0.14
Bicolor	BBrr Bbrr	0.2475	0.0825	0.0000	0.3300	0.33
Nigra	bbrr	0.0000	0.5300	0.0000	0.5300	0.53
Sum		0.3175	0.6825	0.0700	0.9300	1.00

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